CHEMOKINE-BINDING ACTIVITIES OF M3 PROTEIN ENCODED BY MURINE GAMMAHERPESVIRUS 72

P. BELVONČÍKOVÁ[1], A. KRÁĽOVÁ[2], M. KÚDELOVÁ[1], V. HAJNICKÁ[1], I. REŽUCHOVÁ[1], I. VANČOVÁ[1*]

[1]Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic; [2]Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

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Summary. – Murine gammaherpesvirus 68 (MHV-68) contains gene-encoding M3 protein expressed during the acute and persistent phase of infection. This protein features a chemokine-binding activities (Parry *et al.*, 2000; van Berkel *et al*., 2000). In this study, we demonstrated that the Murine gammaherpesvirus 72 (MHV-72) also contained M3 gene with the codon-changing mutation at the position 920 nt converting amino acid (aa) 307 Asp (GAC) to Gly (GGC). The mutation in the M3 protein was localized near chemokine-binding domain and was able to change the secondary structure of M3 protein. We examined the binding activities of M3 proteins of MHV-72 and MHV-68 to five human chemokines (CCL3, CCL5, CCL11, CCL2, and CXCL8). Binding activity of MHV-72 M3 protein to CCL5 as well as to CXCL8 reached only 11.1% (day 3 p.i.) to 20% (day 4 p.i.) of the activity detected for MHV-68 M3 protein. On the other hand, MHV-72 M3 protein bound to human cytokines CCL11 and CCL2 reached about 90% of the binding detected for MHV-68 M3 protein. The binding activity of both M3 proteins to human CCL3 was similar. These data implied that mutation identified in MHV-72 M3 protein might be involved in attenuation of immune response to infection with MHV-72.

Key words: murine gammaherpesvirus; chemokine-binding protein; M3 protein

Introduction

Chemokines are a family of small structurally related proteins produced and released by a wide variety of cell types. They direct leukocyte migration from blood vessels to sites of infection or tissue injury. On the basis of the arrangement of cystein residues at their N-terminus, they fall into four subfamilies: CXC, CC, C, and CX_3C (Zlotnik and Yoshie, 2000). The specific effects of chemokines on their target cells are mediated by the members of a family of 7-transmembrane-spanning, G-protein coupled receptors. As largely basic molecules, chemokines interact also with negatively charged glycoaminoglycans (GAGs) expressed at the endothelial cell surface. These interactions are involved in the chemokine transport and presentation (Middleton *et al*., 2002).

Chemokines play a crucial role in the host defense. They are involved in both innate and adaptive immunity (Luster, 2002). Not surprisingly, many infectious agents developed strategies to manipulate host chemokine network. Large DNA viruses, such as poxviruses and herpesviruses, encode viral protein homologs of chemokines (vCK), viral protein homologs of chemokine receptor (vCKR) and viral chemokine-binding proteins (vCKBP) that neutralize chemokine activity (Lalani *et al*., 2000).

*Corresponding author. E-mail: viruvan@savba.sk; fax: +4212-54774284.

Abbreviations: CCL5 = regulated upon activation, normal T cell expressed and secreted, RANTES; CCL2 = monocyte chemoattractant protein 1, MCP-1; CCL3 = macrophage inflammatory protein 1 alpha, MIP-1a; CCL11 = eotaxin; CTD = C-terminal domain; CXCL8 = interleukin-8; EBV = Epstein-Barr virus; GAGs = glycoaminoglycans; HHV-8 = human herpesvirus 8; MHV-68 = Murine gammaherpesvirus 68; MHV-72 = Murine gammaherpesvirus 72; NTD = N-terminal domain; p.i. = post infection; RT = room temperature; vCK = viral protein homologs of chemokines; vCKBP = viral chemokine-binding proteins; vCKR = viral protein homologs of chemokine receptors

Viral proteins with chemokine-binding abilities are unique products with no sequence similarity between themselves or with host chemokine receptors. The first herpesvirus protein able to bind chemokines was M3 protein found in MHV-68, labeled as vCKBP3 (Parry *et al*., 2000; van Berkel *et al*., 2000). M3 protein (M_r = 44 K) binds chemokines from all four subfamilies. It blocks the interaction of chemokines with their cellular receptors and also the induction of intracellular signaling. In addition, M3 protein can block the interactions of chemokines with GAGs (Webb *et al*., 2003).

MHV-68 (*Murid herpesvirus 4*) belongs to the genus *Rhadinovirus,* the subfamily *Gammaherpesvirinae* (van Regenmortel *et al.*, 2000). It is a natural pathogen of murid rodents genetically related to the primate gammaherpesviruses – Herpesvirus saimiri, Human herpesvirus 8 (HHV-8), and Epstein-Barr virus (EBV). Besides MHV-68, seven more isolates (MHV-60, MHV-72, MHV-76, MHV-78, MHV Šumava, MHV-4556, and MHV-5682) were obtained in Slovak and Czech Republic (Blaškovič *et al*., 1980). To date, the isolate MHV-72 was characterized as to its biological properties (Mistríková *et al*., 2000, 1996; Rašlová *et al*., 2001; Mistríková and Mrmusová, 1998) and also the differences in the sequences of some genes relating to MHV-68 were described (Mačáková *et al*., 2003). More recently, the unique nucleotide mutation in MHV-72 MK3 gene changing the aa at the C-terminus of MK3 protein predicted to interact with TAP1/2 was identified (Valovičová *et al*., 2006).

In this study, we characterized the sequence of MHV-72 M3 gene and the properties of M3 protein secreted into cultivation medium of cells infected with MHV-72. We examined and compared the binding activities of 5 human chemokines (CCL3, CCL5, CCL11, CCL2, and CXCL8) to MHV-72 and MHV-68 M3 proteins.

Materials and Methods

Cells and viruses. Baby hamster kidney (BHK-21) cells were grown in Leibowitz medium L-15 (Gibco) supplemented with 10% (v/v) of fetal bovine serum, 2 mmol/l glutamine (Invitrogen) and 100 mg/ml penicillin-streptomycin-amphothericin (Cambrex). Stock of MHV-68 was kindly provided by Prof. J. Mistríková (Comenius University, Slovak Republic). Purified clone h3.7 of MHV-72 (Rašlová *et al*., 2000) was obtained from Dr. M. Kúdelová (Institute of Virology, Slovak Republic). Virus stocks were prepared by infection of BHK-21 cells at a multiplicity of infection (MOI) 0.1 PFU/cell. Titer of the virus was quantified by plaque assay on BHK-21 cells.

Purification of viral DNA. MHV-72 or MHV-68 DNA was prepared from virions purified on linear sucrose gradient as previously described (Mačáková *et al*., 2003). Purified virions were resuspended in 2x NPE buffer (0.2 mol/l NaCl, 20 mmol/l $Na_2HPO_4.2H_2O$, 1 mmol/l NaH_2PO_4, 2 mmol/l EDTA, pH 7.5), incubated with RNAase A (100 µg/m1) for 30 mins at 37°C and treated for 4 hrs at 56°C with proteinase K and SDS (100 µg/ml and 1%, respectively). The DNA was extracted twice by phenol-chloroform, precipitated by ethanol and dissolved in TE buffer (0.01 mol/l Tris-HCl, 1 mmol/l EDTA, pH 8.0).

M3 gene PCR amplification. To amplify the genomic region of MHV corresponding to the ORF M3, the primer M3CF (5'-GTGGTGCAGACCTCATGGTAC-3') and M3BamF (5'-GCAGGATCCCATGGCCTTCCTATCCACATC-3') were used. Primers were derived from M3 sequence of the prototype MHV-68 (Virgin *et al*., 1997). The PCR mixture contained about 20 ng of MHV-72 or MHV-68 DNA as a template, 0.3 mmol/l dNTPs, 2 mmol/l $MgCl_2$, 0.3 mmol/l of each primer, and 3 U of Taq polymerase (Finnzymes). To exclude the possible involvement of faulty incorporation of nucleotides during PCR reaction, the high fidelity DNA polymerase (DyNAzymeEXT, Finnzymes) was used. The PCR reaction was performed by using Mastercycler personal (Eppendorf) as follows: an initial denaturation at 95°C for 5 mins, followed by 35 cycles at 95°C for 1 min, 54°C for 45 secs, 72°C for 1 min, and finally one cycle at 72°C for 7 mins. The PCR product overlapping the 1342 bp long fragment with either of MHV-72 M3 or MHV-68 M3 gene was purified using Wizard DNA Clean-up System (Promega) and cloned into pGEM-T-Easy vector (Promega). pGEM-T M3 clones were purified using QIAprep Spin Midiprep kit (QIAGEN) and digested with *Eco*RI and *Sph*I to check the length, specificity, and orientation of the fragment cloned. Then, two clones containing MHV-72 M3 gene were selected and sequenced in both directions.

Sequencing of M3 gene. Nucleotide sequence of MHV-72 M3 gene was determined in two independent reactions. We used Big Dye Terminator Cycle Sequencing Ready Reaction with AmpliTaq DNA polymerase (Applied Biosystems) in PCR procedure (20 cycles of denaturation at 96°C for 10 secs, annealing at 50°C for 50 secs, and elongation at 60°C for 1 min) and then the products were purified using DyeEx 2.0 Spin Kit (QIAGEN). Alternatively, we used BigDye Terminator 3.1 Cycle Sequencing Kit. Two universal pUC/M13 forward (24-mer) and reverse (22-mer) primers as well as cloning forward (M3CF) and reverse (M3BamF) primers were used. After electrophoresis on the ABI PRISM 377 DNA Sequencer (Perkin Elmer) or ABI PRISM 3700 DNA Analyzer, the sequences were evaluated by the use of Sequence Analysis 3.3 software. Finally, the sequence of MHV-72 M3 gene was compared with sequence of MHV-68 M3.

Evaluation of sequencing results. Secondary structure predictions for MHV-72 M3 or MHV-68 M3 proteins were calculated using the method of Garnier-Osguthorpe-Robson (Garnier *et al*., 1978). M3 proteins were also analyzed for hydrophilicity and surface exposure. Predictions were calculated using methods derived from Kyte and Doolittle (1982), Hopp and Woods (1981), and Janin *et al*. (1978).

Detection of chemokine-binding activities by ELISA. Subconfluent BHK-21 cells grown in L15 medium were infected by MHV-68 or MHV-72 at MOI = 5 PFU/cell. For detection of the chemokine-binding activities, aliquots of cultivation fluid were harvested at 2, 3, and 4 day p.i. The aliquots were clarified by centrifugation (1000 x g for 10 mins) and viral particles in supernatants were inactivated by exposure to UV light for 15 mins at room temperature (RT).

The supernatants from uninfected cells were prepared in the same way and used as a negative control. Cultivation fluids from

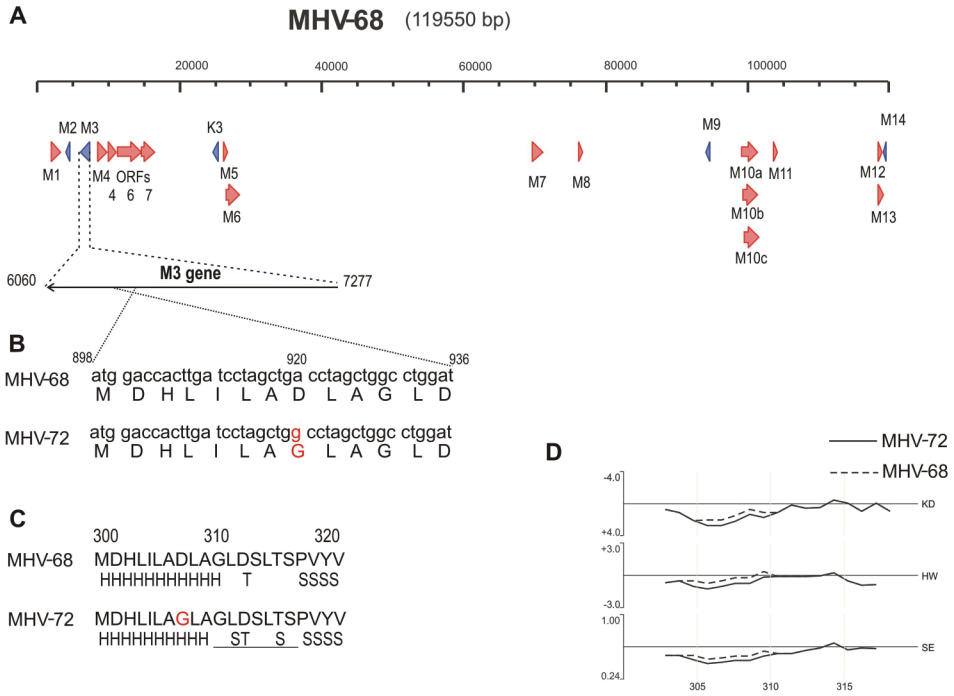

Fig. 1

Analysis of mutation in MHV-72 M3 gene

(A) Localization of ORF M3 gene and the specific (M1-M14) genes within MHV-68 genome; (B) Primary structure of partial sequence of M3 gene (from nt 898 to 936) with MHV-72 specific mutation at nt 920 causing codon change within the M3 protein sequence; (C) Predicted secondary structure of sequence from aa 300 to 320 of MHV-72 and MHV-68 M3 proteins. The upper line is aa sequence using the standard one-letter code. The lower line is the predicted secondary structure using Garnier-Osguthorpe-Robson analysis (H-helix, T-turn, S-sheet). The changes in secondary structure are underlined; (D) Comparative hydrophilicity analysis of CTD region of MHV-72 (—) and MHV-68 (---) M3 proteins (from aa 300 to 320, mutation at aa 307) analyzed by Kyte and Doolittle (KD) and Hopp and Woods analysis (HW). Values above the axis line are hydrophilic and values below are hydrophobic. Surface exposure analysis (SE) showed the peak values above the axis line as exposed on the surface of the protein.

MHV-68-infected cells were used as a positive control. The levels of chemokine-binding activities of inactivated viral samples were measured using ELISA kits for 5 human recombinant chemokines (R&D Systems) unless indicated otherwise: human CCL3/MIP-1 alpha DuoSet, human CCL5/RANTES DuoSet, human CCL11/ Eotaxin DuoSet, human CXCL8/IL-8 Module Set, and human CCL2/MCP-1 Module Set (Bender MedSystems Diagnostics). For each assay, 50 pg of recombinant chemokine in diluent were mixed with 10 µl of cultivation fluid to give total volume of 105 µl. Each mixture was incubated for 1.5 hr at RT with gentle shaking and then applied to the ELISA plates (100 µl/well). Absorbance measurements were recorded in the linear range of the standard curves for each ELISA kit in duplicates. A reduction in detectable levels of a particular chemokine, when compared with the 50 pg of recombinant chemokine as a control was interpreted as an evidence of chemokine-binding activity of viral sample. The results, expressed as mean +/–SEM of two independent experiments, constitutes percentage reduction of OD reading compared with the control.

^{125}I-CCL2 binding to viral samples. The 20 µl of viral samples – equivalent to cultivation fluids of 2 x 10^5 BHK-21 cells infected with MHV-68 or MHV-72 at MOI = 5, were incubated for 90 mins at RT with 2 µl of human recombinant ^{125}I-CCL2 [specific activity ~74 TBq/mmol, (63,000 cpm/vial), Amersham Biosciences]. The protein complexes were covalently cross-linked by addition of 4 µl of 50 mmol/l BS3 (Bis-SulfosuccinimidylSuberate, Pierce) for 30 mins at RT and the chemical cross-link reaction was terminated by 1.5 µl of 1 mol/l Tris-HCl (pH 7.2). Products were analyzed by SDS-PAGE using 12.5% gels and autoradiography.

Results

Sequence analysis of the MHV-72 M3 gene

The sequence of MHV-72 M3 gene is 1221 bp long (Acc. No. DQ378056) (Fig. 1A). A comparison of MHV-72 and MHV-68 M3 genes (Acc. No. AF127083) showed one codon changing mutation at nt position 920 (change A to G) converting aa 307 Asp (GAC) to Gly (GGC) localized near the chemokine-binding site (Fig. 1B). Mutation identified in the MHV-72 M3 gene caused changes in the predicted secondary structure of the MHV-72 M3 protein (Fig. 1C) resulting in a lowered hydrophilicity and also surface

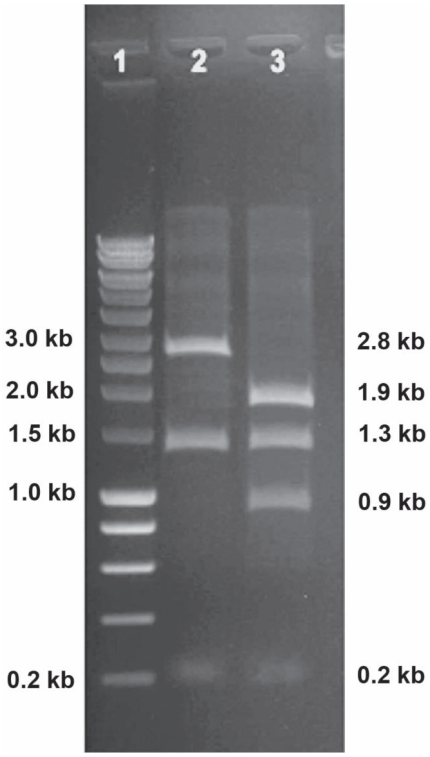

Fig. 2

Detection of specific mutation in MHV-72 M3 gene by restriction analysis with *Bgl*I

1kb DNA ladder (lane 1); pGEM-T-M3 MHV-68 plasmid (4.38 kb) produced three fragments 2.8, 1.3, and 0.2 kb (lane 2); pGEM-T-M3 MHV-72 plasmid (4.38 kb) produced four fragments 1.9, 1.3, 0.9, and 0.2 kb (lane 3).

exposure of region involved in chemokine-binding (Fig. 1C,D). This finding allowed us to predict the changed properties of the gene product of MHV-72 M3.

In addition, the mutation in the M3 gene created a new restriction site for *Bgl*I endonuclease for a clear-cut identification of the isolate MHV-72 (Fig. 2).

The chemokine-binding activities of MHV-72 M3

To evaluate the time of infection with MHV-72 that was required for the production of viral M3 chemokine-binding protein, the cultivation fluids from MHV-72- and mock-infected cells were collected at day 2, 3, and 4 p.i. We examined the chemokine-binding activity of M3 protein that consequently inhibits an ability of chemokine to combine with its specific antibody. M3 chemokine-binding activities identified in cultivation fluids of both MHV-72 and MHV-68-infected cells revealed the dependence on the time of the infection. The chemokine-binding activities in the cultivation fluids of MHV-72-infected cells were detected on day 3 p.i.,

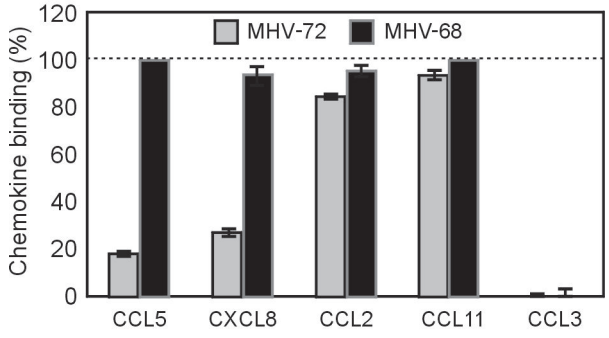

Fig. 3

Chemokine-binding activities of MHV-72 and MHV-68 M3 proteins

The human chemokines CCL5, CXCL8, CCL2, CCL11, and CCL3 binding was examined in ELISA at day 4 p.i.

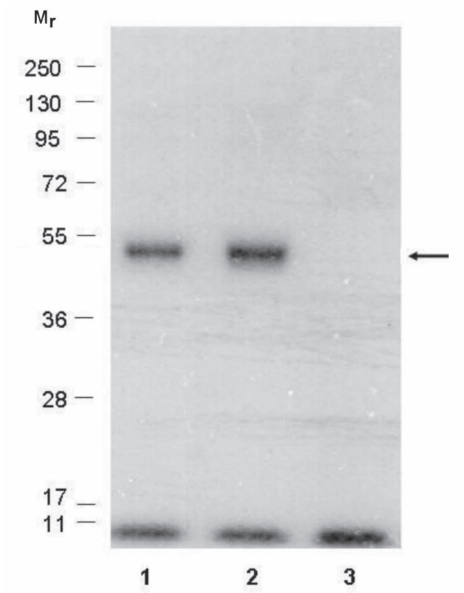

Fig. 4

SDS-PAGE of MHV-72 and MHV-68 M3 proteins bound to ^{125}I-CCL2

Protein complexes prepared from MHV-72-infected (lane 1) and MHV-68-infected (lane 2) cultivation fluids. Mock-infected cultivation fluid contained only unbound ^{125}I-CCL2 (8.7 K)(lane 3). Arrow indicates position of 53 K protein complex. M_r standards on the left.

whereas the activities of MHV-68-infected fluids were measurable on day 2 p.i. (data not shown). The highest chemokine-binding activities were detected on day 4 p.i. for both MHV-72 and MHV-68 M3 proteins (Fig. 3).

The major difference between MHV-72 M3 vs. MHV-68 M3 proteins in chemokine-binding activities we detected with CXCL8 and CCL5 chemokines. MHV-72 M3 protein

was able to bind only 27% of CXCL8, but MHV-68 M3 protein bound as much as 94%. In case of CCL5, we observed only 18% of binding activity for MHV-72 M3 protein. However, MHV-68 M3 was able to bind 99% of CCL5. For this chemokine we demonstrated the highest difference between MHV-72 M3 and MHV-68 M3 binding activities (Fig. 3).

In addition, we observed slight difference in binding activities for CCL2. The MHV-72 M3 was able to bind 85% of CCL2, but MHV-68 M3 bound 95%. The highest chemokine-binding activities of both M3 proteins were detected for CCL11. The MHV-72 M3 protein bound 93% of CCL11 and the MHV-68 M3 protein 99%. For this chemokine only slight difference between MHV-72 M3 and MHV-68 M3 binding activity was found. Almost undetectable binding activities for CCL3 chemokine (0.375% versus 0.34 %) were found for both M3 proteins.

Summing up, MHV-72 M3 protein present in cultivation fluids of infected cells showed lower chemokine-binding activities than MHV-68 M3 protein.

The M3 protein of MHV-72 binds ^{125}I-CCL2

To confirm an identity between the chemokine-binding activities present in cultivation fluids of MHV-72-infected cells and the M3 protein, we used a chemical cross-linking between ^{125}I-CCL2 and M3 protein. The resulting protein complexes were examined by SDS-PAGE and auto-radiography. The complex of chemokine and M3 protein was detected in cultivation fluids from MHV-72- and MHV-68-infected cells, but not in the cultivation fluid from mock-infected cells (Fig. 4). The size of the complex was approximately 53 K, what corresponded to M_r of CCL2 (8.7 K) and M3 protein (44 K) combined.

Discussion

Intranasal administration of MHV-68 results in an acute productive infection of lungs alveolar epithelial cells and in a latent infection of several cell types including B lymphocytes, macrophages, and epithelia. The virus infection induces an inflammatory infiltrate in the lungs and enlargement of lymph nodes and spleen, caused by a robust chemokine response (Weinberg *et al*., 2002). The M3 protein, encoded by ORF for M3 gene of MHV-68 was identified as the first example of a soluble inhibitor encoded by a herpesvirus that binds chemokines. The M3 protein binds chemokines of the CC, CXC, CX_3C, and C families with high affinity and prevents chemokine-induced signal transduction in vitro (Parry *et al*., 2000; van Berkel *et al*., 2000). MHV-68 M3 belongs to early-late lytic genes expressed early during the establishment of latency *in vivo* (Simas *et al*., 1999). In this study we described M3 protein encoded by the MHV-72, also labeled as soluble vCKBP3.

Preliminary results of experimental infection with MHV-72 in mice showed that the expression of M3 gene might be dependent on the route of infection. In peritoneal cells of the infected mice, M3 gene transcription appeared continual throughout a lytic and latent infection with MHV-72 (from day 3 to 42 p.i.) and was independent on the route of infection (intranasal or subcutaneous). The M3 gene transcription in spleen was found similar to that in peritoneal cells after intranasal route of infection (from day 3 to 42 p.i.) but delayed and discontinued (at day 10 p.i. and at day 42 p.i., respectively) after subcutaneous infection (M. Kúdelová, personal communication).

Macrophages and dendritic cells of mice infected with MHV-68 are more likely to be a source of M3 *in vivo* than germinal-center B cells. The role of M3 protein in the beginning of latency remains still controversial, but it is possible that lytic infection of macrophages and dendritic cells may indirectly protect latent infection of B cells (Marques *et al*., 2003). In addition to, M3-deficient mutant of MHV-68 was found to lack detectable chemokine-binding activity and was unable to establish the latent infection (Bridgeman *et al*., 2001).

M3 protein appears to establish a wide range of recognition for members of all four chemokine families through the use of conformational plasticity and electrostatic complementation. Further, M3 has evolved a mode of ligand binding that appears to mimic the strategies employed in cognate receptor binding. The crystal structure of MHV-68 M3 protein reveals a novel structure that tightly associates as an anti-parallel dimmer (Alexander *et al*., 2002). M3 is an extremely acidic protein (pI 4.2), with an especially significant clustering of acidic aa residues on the loops projecting from the N-terminal domain (NTD). The chemokine-binding sites of M3 are deep clefts formed between the NTD (aa 1–210) and C-terminal domain (CTD) (aa 211–382) ß sandwiches. The chemokine-binding surface is equally distributed between the two subunits of M3 e.g. 16 aa in the NTD and 13 aa in the CTD that lose surface accessibility, when a complex is formed. The primary structural element of the CTD able to bind chemokine is the A-B loop, which includes the h6 helix and B'-strand (Alexander *et al*., 2002). Furthermore, a hydrophobic pocket formed by the A"-A', A-B, and E-F loops of M3 which serves to sequester CCL2 represents a critical residue for GPCR binding and signaling (Alexander *et al*., 2002). We identified the mutation in the M3 of MHV-72 that lies in the B-strand of former A-B loop at aa 307 (changing Asp to Gly) near to the chemokine-binding interface residues. Predicted secondary structure of the MHV-72 M3 protein confirmed that identified mutation results in lowered hydrophilicity and also surface exposure of close region that allow to predict

changed chemokine-binding properties. The MHV-68 M3 protein has been shown to bind strongly to murine CCL3, murine CCL2 , human CCL5, murine CXCL1 , CX3CL, and human CXCL8, but not to human B cell-specific or murine neutrophil specific CXC chemokines (Parry *et al*., 2000; van Berkel *et al*., 2000).

In this work, we demonstrated a lower affinity of the MHV-72 M3 to human CCL5 and CXCL8 in comparison to MHV-68 M3 in all examined intervals p.i. Binding affinity to CCL5 as well as to CXCL8 detected for the MHV-72 M3 reached only 11.1 to 20% of affinity detected for MHV-68 M3. In case of CXCL8 molecules, study on MHV-68 M3 protein acting by mimics binding of chemokine receptors were provided by Webb *et al*. (2003). Comparison with their results showed that in our experiments the CXCL8-binding activity found for MHV-72 M3 was three times weaker than for MHV-68 M3.

On the other hand, the MHV-72 M3 protein has been shown to bind to human CCL11 and CCL2 with high affinity reaching more than 90% of the affinity of MHV-68 M3 protein. Moreover, we demonstrated very strong chemokine-binding activity for CCL11, a member of chemokines that selectively attracted Th2 lymphocytes expressing CCR4 and CCR8 on their surface, for both MHV-72 M3 and MHV-68 M3 proteins.

Finally, we investigated the binding activity of MHV-72 M3 protein to human CCL3 that is required for natural killer cells trafficking (Salazar-Mather *et al*., 2000). This activity was either for MHV-72 M3 or MHV-68 M3 very low (about 1%). In contrast, Parry *et al*. (2000) detected measurable affinity of MHV-68 M3 to the murine CCL3. It may reflect species differences between mammalian chemokines tested or differences between M3 proteins produced by MHV-infected mammalian cells and recombinant M3 protein produced by insect cells.

M3 protein produced by MHV-infected cells may alter migration of CCR7-expressing T, B, or dendritic cells toward local gradients of CCL19 and CCL21 and disrupt immune response. By blocking the CCL21/CCR7-dependent migration of dendritic cells to the lymph node, M3 could potentially delay initiation of specific immune response against MHV-68 (Jensen *et al*., 2003). An upregulation of control trafficking of lymphocytes and macrophages in CNS during MHV-68 infection was demonstrated (van Berkel *et al*., 2002). The direct correlation between M3 expression and inhibition of lymphocyte and macrophage trafficking in CNS was also established (Jensen *et al*., 2003). In this context, many authors consider CCL2 as a key factor in the host defense mechanism against virus infection in CNS. We demonstrated very strong CCL2 chemokine-binding activity for MHV-68 M3 (95%) and MHV-72 M3 (nearly 85%) proteins. Taken together, besides binding activity to CCL11, the binding activity to CCL2 was the second highest binding activity of MHV-72 M3 comparable with MHV-68 M3.

Potential attenuation of immune response to MHV-68 infection by M3 protein could lead to the reduction of leukocytes recruitment to the lungs. This could lead to enhanced viral replication. However, MHV-68 is able to induce a substantial inflammatory infiltrate in the lungs and many of the infiltrating cells are activated T cells, which are required for viral clearance. However, an abundant expression of M3 does no affect the titers of infectious virus in the lungs and the level of chemokines CCL5, CCL11, CCL2, CCL3, CCL4 and CXCL10 expressed in the lungs of MHV-68-infected mice. However, M3 may significantly inhibit the effector's functions of chemokines in smaller micro-environment within lungs (Sarawar *et al*., 2002). This action would provide a selective advantage for the virus by inhibiting the antiviral activity of recruited leukocytes allowing the virus to replicate at higher levels in presence of cells serving as potential targets for establishment of latency. Probably, it may be that tissue damage at least during the initial stages of acute phase replication in the lungs, results in such abundance of chemokines that M3-mediated blockade is overwhelmed. Lytic replication is notably lacking in MHV-68-infected lymphoid tissue and the lack of tissue damage and consequent innate immune activation in this site may be crucial for successful immune evasion by M3 (Bridgeman *et al*., 2001).

Although several data describing gene sequences differences between MHV-68 and MHV-72 are available (Mačáková *et al*., 2003; Valovičová *et al*., 2006) studies focused on pathogenetical differences of both strains are still rare. However, MHV-68 is intensively studied in many laboratories as a model for human gammaherpesvirus infection and is suggested as more pathogenic and less oncogenic as MHV-72 (Nash *et al*., 2001; Mistríková and Mrmusová, 1998; Mistríková *et al*., 1996).

In this report, M3 protein encoded by MHV-72 has been shown as similar, but not identical with the M3 protein of MHV-68 at least in the binding activity to CCL5 and CXCL8. To reveal the role of M3 protein in potentially lower attenuation of immune response to infection with MHV-72 needs further investigation.

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